10/531971
PCT/KR2003/002704
Rec'd PCT/PTO 20 APR 2005

MICROORGANISM PRODUCING 5'-XANTHYLIC ACID

Technical Field

The invention relates to a microorganism producing 5'-xanthylic acid. More particularly, the invention relates to selecting a mutant strain of *Corynebacterium ammoniagenes* KCCM 10340 in order to enhance growth activity, which can rapidly overcome growth-standing phase on early culture and can accumulate 5'-xanthylic acid in culture medium for same period of fermentation at a high yield and high concentration rate.

Background Art

5'-xanthylic acid is an intermediate in the nucleic acid biosynthesis process, which is physiologically important in the body of animals and plants, used in food, medical supplies and other various field. The invention relates to a mutant strain from a known strain *Corynebacterium ammoniagenes* KCCM 10340, producing 5'-xanthylic acid by a direct fermentation method at a high yield and high concentration rate than existing technique.

5'-xanthylic acid is an intermediary product of purine nucleotide biosynthesis process and important material for producing 5'-guanylic acid. A widely used method to produce 5'-guanylic acid having fineness and high quality is microorganism fermentation method which produces 5'-xanthylic acid first and converts it into 5'-guanylic acid enzymatically, therefore, to produce 5'-guanylic acid, corresponding amount of 5'-xanthylic acid is necessary. Conventional methods to produce 5'-xanthylic acid are chemosynthesis, deaminization of 5'-guanylic acid which is produced as a result of decomposition of ribonucleic acid in yeast, a fermentation method to add xanthine as precursor material in fermenting medium, a fermentation method to use a mutant strain of

microorganism, a method to add antibiotic material (JP 1477/42 and JP 20390/44), a method to add surfactant (JP 3825/42 and JP 3838/42) and so on. Among these, a direct fermentation method of 5'-xanthylic acid by a mutant strain of microorganism is quite advantageous in terms of industrial aspect. Thus, we inventors developed a mutant strain with increased productivity of 5'-xanthylic acid, by modifying the existing character of *Corynebacterium ammoniagenes* KCCM 10340 into the character of producing 5'-xanthylic acid at a large yield rate.

Most microorganisms reach to the condition that the volume doesn't increase any more when keep on culturing under the constant condition, and especially the concentration of microorganism producing primary metabolite, a growth-dependent product, doesn't increase any more. It is mainly caused by limited supply of dissolved oxygen. Method of enhancing aeration and agitation condition, for removal of the limited supply of dissolved oxygen, is used, but there is technical and economical limit in actual production method. To overcome the limit and increase yield rate and concentration of 5'-xanthylic acid by enhancing the volume of microorganism and various physiological activity, under the limited supply of dissolved oxygen, we inventors thought that the method of producing the novel microorganism showing superior growth under the same condition, would be useful. Thus, the inventors investigated and found out that a mutant strain, which can rapidly overcome growth-standing phase on early and shows superior growth, is most effective, and can produce 5'-xanthylic acid at a high yield and high concentration rate than existing technique, and accomplished in this invention.

Disclosure of the Invention

The invention relates to *Corynebacterium ammoniagenes* CJXSP 0201

(KCCM-10448) which is a mutant strain of *Corynebacterium ammoniagenes* KCCM 10340, producing 5'-xanthylic acid.

The CJXSP 0201 is obtained by adapting *Corynebacterium ammoniagenes* KCCM 10340 as parent strain by spontaneous mutation method and selecting a mutant strain from them. The spontaneous mutation method is the following. 5mL of nutrient medium (glucose 20g/L, peptone 10g/L, yeast extract 10g/L, sodium chloride 2.5g/L, urea 3g/L, adenine 150mg/L, guanine 150mg/L, pH 7.2) was poured into a test tube having diameter of 18mm and sterilized under pressure according to the common methods. Then, *Corynebacterium ammoniagenes* KCCM 10340 was seeded into and it was cultured with shaking at 200rpm, 30℃ for 18 hours and the resultant was used as seed culture. 50㎕ of the seed culture was seeded into 500mL-Erlenmeyer flask for shaking which had been sterilized and 40mL of minimum medium (glucose 20g/L, potassium phosphate monobasic 1g/L, potassium phosphate dibasic 1g/L, urea 2g/L, ammonium sulfate 3g/L, magnesium sulfate 1g/L, calcium chloride 100mg/L, ferrous sulfate 20mg/L, manganese sulfate 10mg/L, zinc sulfate 10mg/L, biotin 30㎍/L, thiamine hydrochloride 0.1mg/L, copper sulfate 0.8mg/L, adenine 20mg/L, guanine 20mg/L, pH 7.2) had been added in. Then, it was cultured with shaking at 200rpm, 30℃ for 24 hours, and when it reached to early log phase of growth, 50㎕ of the culture was seeded into another 500mL-Erlenmeyer flask for shaking in which 40mL of the minimum medium had been added. And when it reached to early log phase of growth (Optical Density 0.5 (λ=562nm)) again, 50㎕ of the culture was seeded into another 500mL-Erlenmeyer flask for shaking in which the minimum medium had been added again. Such a process, namely subculture was repeated 20 times. The final culture was streaked on petri-dish of the minimum medium containing 1.5% agar and was cultured in 30℃ incubator until colony formed. Among the colonies, colonies showing rapid growth rate

relatively were selected as superior mutant strain. And from them, a strain which shows superior 5'-xanthylic acid productivity and growth rate, was separated, named CJXSP 0201, and it was deposited under Budapest Treaty to the Korean Culture Center of Microorganisms on November 21, 2002 with accession Number KCCM 10448. The time for colony forming is 38 hours in KCCM 10340, a known strain, while CJXSP of the invention 0201 takes 31 hours, therefore CJXSP is a mutant strain having character of superior growth.

BEST MODE FOR CARRYING OUT THE INVENTION

Example 1

Used strains: *Corynebacterium ammoniagenes* KCCM 10340, *Corynebacterium ammoniagenes* CJXSP 0201

Seed medium: glucose 30g/L, peptone 15g/L, yeast extract 15g/L, sodium chloride 2.5g/L, urea 3g/L, adenine 150mg/L, guanine 150mg/L, pH 7.2

Fermentation medium: (1) A medium: glucose 60g/L, magnesium sulfate 10g/L, ferrous sulfate 20mg/L, zinc sulfate 10mg/L, manganese sulfate 10mg/L, adenine 30mg/L, guanine 30mg/L, biotin 100μg/L, copper sulfate 1mg/L, thiamine hydrochloride 5mg/L, calcium chloride 10mg/L, pH 7.2

(2) B medium: potassium phosphate monobasic 10g/L, potassium phosphate dibasic 10g/L, urea 7g/L, ammonium sulfate 5g/L

Fermentation method: 5mL of the seed medium was poured into a test tube having diameter of 18mm and sterilized under pressure according to the common methods. After the sterilization, a *Corynebacterium ammoniagenes* KCCM 10340 and *Corynebacterium ammoniagenes* CJXOL 0201 were seeded in respectively

and it was cultured with shaking at 180rpm, 30℃ for 18 hours. The resultant was used as seed culture. Then, as fermentation medium, A medium and B medium were sterilized separately under pressure according to the common methods and 29mL of A medium and 10mL of B medium were respectively poured into sterilized 500mL-Erlenmeyer flask for shaking and 1mL of the above-mentioned seed culture was seeded into and fermented at 200rpm, 30℃ for 90 hours. After the fermentation was completed, the amount of accumulation of 5'-xanthylic acid in the medium showed that the amount in KCCM 10340 was 23.0g/L and the amount in CJXSP 0201 was 24.7g/L. (The concentration of accumulated 5'-xanthylic acid is given by 5'-sodium xanthate·$7H_2O$.)

Example 2

Used strains: same as example 1.

Primary seed medium: same as the seed medium of example 1.

Secondary seed medium: glucose 60g/L, potassium phosphate monobasic 2g/L, potassium phosphate dibasic 2g/L, magnesium sulfate 1g/L, ferrous sulfate 22mg/L, zinc sulfate 15mg/L, manganese sulfate 10mg/L, copper sulfate 1mg/L, calcium chloride 100mg/L, biotin 150μg/L, adenine 150mg/L, guanine 150mg/L, thiamine hydrochloride 5mg/L, antifoaming agent 0.6mL/L, pH 7.2

Fermentation medium: glucose 151g/L, phosphoric acid 32g/L, potassium hydroxide 25g/L, adenine 198mg/L, guanine 119mg/L, ferrous sulfate 60mg/L, zinc sulfate 42mg/L, manganese sulfate 15mg/L, copper sulfate 2.4mg/L, alaniate 22mg/L, NCA 7.5mg/L, biotin 0.4mg/L, magnesium sulfate 15g/L, cystinate 30mg/L, histidinate 30mg/L, calcium chloride 149mg/L, thiamine hydrochloride

15mg/L, antifoaming agent 0.7mL/L, CSL 27mL/L, tuna extract 6g/L, pH 7.3

Primary seed culture: 50mL of the primary seed medium was poured into 500mL-Erlenmeyer flask for shaking and sterilized under pressure at 121℃ for 20 minutes. After cooling, *Corynebacterium ammoniagenes* KCCM 10340 and *Corynebacterium ammoniagenes* CJXOL 0201 were seeded into respectively and it was cultured with shaking at 180rpm, 30℃ for 24 hours.

Secondary seed culture: The secondary seed medium was poured into 5L-experimental fermentation baths (2L each) and sterilized under pressure at 121℃ for 10 minutes. After cooling, 50mL of the above primary seed culture was seeded and cultured with the air supply of 0.5vvm, at 900rpm, 31℃, for 24 hours. During the culturing process, the pH level of the medium was kept at 7.3 with adjusting by ammonia solution.

Fermentation method: The fermentation medium was poured into 30L-experimental fermentation baths (8L each) and sterilized under pressure at 121℃ for 20 minutes. After cooling, the above secondary seed culture was seeded into (1.5L each) and cultured with the air supply of 1vvm, at 400rpm, 33℃. Whenever the residual sugar level drops below 1% during the culturing process, sterilized glucose was supplied and the total sugar level in the fermentation medium was kept at 30%. During the culturing process, the pH level of the medium was kept at 7.3 with adjusting by ammonia solution and the process took 90 hours. After the fermentation was completed, the amount of accumulation of 5'-xanthylic acid in the medium showed that the amount in KCCM 10340 was 137.2g/L and the amount in CJXSP 0201 was 146.8g/L. (The concentration of accumulated 5'-xanthylic acid is given by 5'-sodium xanthate·$7H_2O$.)

Industrial Applicability

The invention adopted *Corynebacterium ammoniagenes* KCCM 10340 as parent strain and treated it UV radiation, mutation derivatives such as N-methy-N'-nitro-n-nitrosoguanidine (NTG) according to ordinary procedure. The KCCM 10340 strain has a resistance to osmotic pressure, caused by high concentration of 5'-xanthylic acid accumulated during culturing process, high concentration of glucose and various carbon source added into culture medium, which results in the high osmotic pressure outside bacterial body, inhibition of normal physiological activity of 5'-xanthylic acid-producing cell and decrease of 5'-xanthylic acid production. In order to obtain a strain having enhanced character of growth activity under the same condition, the invention modified *Corynebacterium ammoniagenes* KCCM 10340 and selected a mutant strain which can rapidly overcome growth-standing phase on early culture, and made it possible to accumulate 5'-xanthylic acid in culture medium at a high yield and high concentration rate for same period of fermentation.